

New Method of Tritium Labeling of
Pure Compounds and Coal Derivatives*

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Introduction

The primary broad objective of this work was to develop the best generalized method of labeling organic materials by exchanging hydrogen with tritium. Of more particular interest was the production of radio-tracers by application of the best tritium labeling method to coal and to products derived from coal by hydrogenation or other processes. These tracers would ultimately be for studies of the mechanism of physical and chemical processes in which these materials are used.

Generally, the materials that are to be labeled for coal processing research are of very complex and usually unknown chemical structure. In such cases, labeling with carbon-14 is precluded since the only method available for incorporating this polyvalent isotope is chemical synthesis. On the other hand, potentially any organic material can be tagged with tritium since the universally present monovalent hydrogen is susceptible to isotopic exchange under proper conditions. This applies to non-descript organic materials as well as pure compounds.

The most important qualification of any labeling method is that it yields tracers of chemical structure identical with the material being labeled. Any production of tagged by-products of altered chemical structure requires stringent post-labeling purification before trustworthy tracers would be obtained.

Two general methods of tritium tagging have been evaluated. The first evaluation was of the more familiar Wilzbach method, which induces self-labeling by the beta radiation from tritium gas in contact with the material to be tagged. Numerous difficulties were found in this method of producing tracers, mostly arising from radiolysis damage to the tracer, as will be pointed out later. Dissatisfaction with the Wilzbach method led to development of a new method, based upon a highly reactive tritiating reagent that promises wide applicability. This latter method utilizes catalytic activity to promote isotopic exchange of tritium into the tracer, as opposed to the radiation induced exchange of the former method with its inherent molecular damage.

Both labeling methods have been tested on hydrocarbons that typify chemical structures expected in coal and coal derivatives, to provide background information for tagging these materials. This is still considered an interim report, in the sense that much more extensive work is indicated to determine the variety of compounds, other than hydrocarbons, that can be

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successfully labeled with the tritiating reagent method. It is conceivable that this method may be sufficiently universal to apply, not only to a wide array of coal and petroleum materials, but also to some pharmaceutical and biological compounds.

Though much work has been done in the past on the use of acids and acid catalysts to promote hydrogen isotopic exchange, especially with deuterium, the past methods have several disadvantages. Some isotopic exchange is obtained with powerful inorganic acids as concentrated sulfuric. However, it cannot be considered as a generalized isotopic exchange reagent because of undesirable sulfonation reactions with aromatic compounds. Also, the exchange rate is often not very fast. Weaker acids as phosphoric require elevated temperatures to effect a reasonable rate of exchange. Under these conditions, undesirable side reactions as formation of tars and polymers are observed for aromatic hydrocarbons.

We then considered boron trifluoride as catalyst to promote isotopic exchange, with tritiated phosphoric acid as the tritium carrier. The boron trifluoride forms a one-to-one molar complex with phosphoric acid and it was soon observed that this complex was a powerful tritiating reagent, free of side product generation for many tested hydrocarbons.

Experimental Procedures

General

Three methods of assay of the specific activity of tritium in tagged samples were examined. The zinc fusion - gas ion chamber method was tried initially. The method has been described in detail by Wilzbach^{1,2} and by Tolbert³. The total sample is gasified to a mixture of mostly H₂ and CH₄ and the radioactivity of the gasified material determined in a gas ion chamber as described by Tolbert³. The ion current is measured with an electrometer. This method was satisfactory for materials of high activity, of 100 uc/gm or more. It did not have sufficient sensitivity for accurate assay of low activities such as 1 to 0.1 uc/gm because of extraneous fluctuating background readings.

The second method tried was direct liquid scintillation counting of the sample. A single channel counting apparatus was assembled as described by Hodgson and Gordon⁴. A special low, dark current, low noise photo multiplier tube (Type 9536A, EMI Electronics Ltd., England) was used in a cold box at -10°C for sensing the scintillations. Attached electronic counting apparatus consisted of a preamplifier and commercial scaler. The method was satisfactory for relatively few pure colorless liquids. Many of our materials, either natural or model compounds, are colored or highly quenching in the liquid scintillators and cannot be counted this way.

The final method adopted is applicable to all types of organic products, including highly colored natural materials. The method is one that has been previously recommended by Quinn⁵ and involves dry combustion of the sample followed by liquid scintillation counting of the collected product water by well-established water-counting techniques. The method is more rapid than zinc fusion, up to 20 samples per day can be assayed as opposed to about 4 for the former method. It is sufficiently sensitive for accurate assay of specific

activities down to 0.01 $\mu\text{C/gm}$. Sensitivity could probably be extended another order of magnitude by use of more modern dual channel liquid scintillation counters.

To test for radio-chemical purity of tracers, or conversely, the distribution of tritium among impurities and parent tracer, vapor phase chromatography was used in the manner described by Riesz and Wilzbach⁶. A sample is elutriated through a Perkin-Elmer chromatograph. The effluent from the chromatograph was passed through a thermal conductivity cell and a continuous flow ion chamber in series. A dual chromatograph was thus obtained. The recorded output of the thermal conductivity cell shows the mass associated with each elutriated peak while the recording of the ion chamber current gives the amount of radioactivity associated with corresponding peaks. In some cases, tagged tracers are of low activities, below the sensitivity of the ion-chamber detector. In these cases, the elutriating peaks are collected individually in ice traps and then assayed by combustion and liquid scintillation counting.

Wilzbach Method

The Wilzbach^{1,2,6,7,8} method of labeling has been well described in previous literature. It consists of exposing the material to be tagged to several curies of pure tritium gas in a sealed reaction vessel for several days. Tritium exchange for bound hydrogen occurs under the influence of the beta radiation from the tritium. Unfortunately, besides isotopic exchange, the parent compound undergoes radiolysis such that highly tagged side products are always produced. Thus, it becomes important to determine the distribution of the tritium between the parent unmodified compound and that of the side products of altered chemical structure. This was done according to the chromatography procedure described above. Purification of labeled materials produced by this method was carried out by preparative vapor chromatography as well as ordinary techniques as micro-distillation and crystallization.

$\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ Method

The procedure for making and using the phosphoric acid-boron fluoride tritiating reagent is very simple as indicated in Figure 1. First, tritiated water and phosphorus pentoxide are mixed stoichiometrically to give absolute tritiated phosphoric acid. Then boron fluoride gas is bubbled into the acid until it is saturated (absorption is rapid) which yields a one-to-one ratio of BF_3 to acid, forming the complex shown in Figure 1. This complex reagent is a very dense liquid, immiscible with all hydrocarbons, but soluble in organic compounds containing oxygen. This reagent must never be stored in glassware for extended times. An imperfect seal, allowing long water vapor absorption will lead to hydrofluoric acid attack of the glass sufficiently to dissolve through the container. Polyethylene ware is used for long-term storage as well as for tagging experiments that extend beyond 2 or 3 days. Ordinary glassware can be used with the reagent in experiments lasting a day or two.

The specific activity of tritium in the water used to form the reagent is the primary determinant of the specific activity of the ultimate tracer treated with the reagent. Thus if very high specific activity tracers are desired, high concentrations of tritium oxide are used in making the phosphoric acids.

To tag an organic liquid, it is simply stirred in a round-bottom flask with the reagent. Solids are ground fine and slurried with the reagent. The ratio of reagent to material being tagged is arbitrary, determined by whether one wants to transfer most of the tritium in the reagent to the material or only a little. At equilibrium, the tritium atoms in the mixture are statistically distributed among all exchangeable hydrogen positions, including the three of phosphoric acid. In this preliminary work, we have used a 2 to 1 mass ratio of material being tagged to reagent.

In tagging of coal and coal extract, the material was first ground to -200 mesh before contacting the reagent. The tagged solids were recovered by filtration. The cake was washed three times with water and twice with 1% NaOH solution and again with water until neutral.

After exchange tagging, hydrocarbon liquids as toluene are simply recovered by decantation in a separatory funnel and solids are collected by filtration. If the organic material dissolves in the reagent, as anisole does, then a few drops of water carefully added to the mixture will result in separation of the organic phase for decanting. The tracer is then washed several times with water and dilute sodium bicarbonate to remove all of the reagent and labile tritium.

The radio-chemical purity of the tracers produced with the $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ reagent is demonstrated by examining for any loss in specific activity when the tracers are subjected to three stages of purification. The product is first treated with sodium to eliminate any labile tritium. This is followed by fractional distillation and finally by preparative chromatography with discriminative collection of the single peak identified as the original compound. We have found that these purity tests are more dependable than the dual radiation-mass detection chromatography alone.

Solid materials such as coal extract and coal were only tested for gross physical changes after tagging, such as melting point.

Results

Evaluation of the Wilzbach Tagging Method

A series of pure aromatic hydrocarbons, typical of hydrocarbon types found in coal hydrogenation oils, were tagged by the Wilzbach method. The results obtained are given in Table I. Included are the tests for radio-chemical purity of the labeled products as determined only by vapor phase chromatography with dual radiation and mass detectors. It is noted that the apparent radiolysis damage decreases both with increasing aromaticity and with the number of fused rings. In general, the amount of radiolysis damage seemed encouragingly small for high molecular weights. The method was then likewise applied to the tagging of a series of narrow-boiling fractions of oils derived from coal hydrogenation. Boiling points ranged from 233 to 355°C and the oils are considered as high molecular weight. Here again, the apparent amount of radiolysis damage was quite small. With the exception of one of the middle boiling fractions, the percent tritium in side products seen on the dual chromatographs was five percent or less.

However, such anomalous results were obtained in experiments with the Wilzbach tracers, even with the simple compounds purified by chromatography, that more rigorous examination of radio-chemical purity became imperative.

Naphthalene was chosen for a more stringent investigation of the purity of the Wilzbach tagged material. The naphthalene newly tagged for this investigation appeared, by chromatography, to have even less tagged impurities, i.e., 6.3 percent as opposed to 13.1 percent for the earlier sample shown in Table I. Suspecting hold-up of some radiolysis impurities in the chromatograph, the tritiated naphthalene was run through a fresh column which was then flushed with water vapor. This revealed that only 62% of the radioactivity elutriated with the naphthalene peak, 4% with the light hydrocarbon impurities and 34% was flushed out by the subsequent water. Therefore at least 34% of the tritium was in unstable radiolysis products that were retained by the column. Thus, at best the naphthalene could be only 62% radio-chemically pure. This points out that one should not view radio-chromatography as a sufficient test of radio-chemical purity.

Next, diluted, tritiated naphthalene was run through a series of purifications as shown in Table II. The sodium treatment was suggested by the possibility of labile tritium in radiolysis impurities, that could have transferred to the chromatographic packing (Perkin-Elmer Type A) and then be flushed out by water. Since there is no labile hydrogen in naphthalene, the observed drop in specific activity upon sodium treatment is due to such hydrogen in radiolysis impurities. Recrystallizations slowly but definitely decreased specific activity - further evidence of radio-chemical impurity. Fraction distillation (under vacuum) reduced drastically the specific activity of the center cut of naphthalene, down to about a third of the initial activity. Thus distillation reduced the specific activity more than chromatography, showing that all that elutriates with the naphthalene peak from the chromatograph is not tagged naphthalene. The longer residence time at elevated temperatures in distillation may have removed unstable radiolysis products, such as by polymerization, making distillation a more efficient purifier than chromatography. The question mark in the last row of Table II indicates that even after the shown purification schemes, there is not absolute assurance that the naphthalene is completely radio-chemically pure. In fact, using this final tagged naphthalene in a de-tagging isotopic exchange test with benzene at 380°C resulted in 16.6% loss of tritium from the naphthalene, whereas when tritiated naphthalene produced chemically (and more likely pure) was used under the same conditions, no exchange loss of tritium was observed.

Thus, radiolysis during self-labeling, produces contaminants, in trace chemical amounts but of very high specific activity, which have physical properties so closely resembling the parent compound or so minute in quantity, that they are not distinguished by chromatography and defy all but the most painstaking purifications which may be prohibitively extensive. It follows that but little specific activity remains in the purified tracer.

We are not alone in these observations of purification problems with the Wilzbach method, as several others reported in late 1960 or early 1961. To cite a few others, a group working at the University of Rome⁷ on self-labeling of substituted benzenes and a Bureau of Mines Group⁸ that labeled numerous organics associated with gasoline, had both then observed that careful purification is required. Wilzbach⁹ too has pointed this out - quote⁹ "In view of the number of tritiated impurities which are likely to be present, it is perhaps remarkable that radio-chemical purity can be achieved for any but the simplest of compounds", unquote.

At this point, it was deemed wise to search for another method of tritium labeling, for certainly if simple single compounds are so difficult to obtain radio-chemically pure, it would be practically impossible to completely remove radiolysis contaminants from complex mixtures derived from coal.

Evaluation of the $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ Tagging Method

Initial work was carried out on the evaluation of concentrated sulfuric acid as a reagent for isotopic exchange tagging of tetralin. This method was abandoned when it was found that the formation of sulfonation products could not be avoided and tagging was rather slow. Phosphoric acid was next tried as a reagent. Elevated temperatures of 85°C or higher were required to obtain reasonable tagging rates. At these temperatures, by-product materials were formed by apparent polymerization reactions, invalidating this approach.

All subsequent work was devoted to the use of the tritiated phosphoric acid - boron fluoride complex as the tagging reagent. It was soon found that this material was a very powerful tritiating reagent which overcomes the deficiencies outlined above for other acids, i.e., it is both non-destructive and fast.

Some initial results of tagging with the tritiating reagent are shown in Table III. These compounds were all mixed with half their weight of $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ which had a specific activity of 1400 uc/gm , except for anisole where the reagent had a specific activity of 380 uc/gm . The degree of tagging shown was accomplished in less than 8 hours. It is immediately apparent that the aromatic compounds tag very rapidly as demonstrated by the first five listed in Table III. The non-aromatics that contain a hydrogen on tertiary carbon, namely methylcyclohexane and decalin also tag but at a lesser degree. The non-branched aliphatics as exemplified by cyclohexane and n-octane appear not to tag at all. In fact, fractional distillation and preparative chromatography practically eliminates the tritium activity from these two purified aliphatics. Thus there seems to be some selectivity in this method of labeling, with preferential labeling of aromatic hydrogen positions, considerably less for hydrogen on tertiary carbon atoms and essentially none for non-tertiary alkane hydrogen. Future studies will determine the degree of labeling of other forms of hydrogen as those on carbon atoms adjacent to carbonyl groups and other chemically activated forms.

The radio-chemical purity of the tracers produced with $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ is demonstrated by comparison of the last two columns in Table III and is indeed encouraging. The three purification steps applied were described earlier. The purity results, that is the agreement between the last two columns in Table III, indicate that essentially all the tritium radioactivity is in the radio-chemically pure tracer, recoverable at yields better than 90%. The small tagging observed in the last two compounds - the non-branched aliphatics - is attributable to chemical impurities originally present before tagging. Indications are that in most cases, if one starts with an absolutely pure compound, after-purification of the tracer is unnecessary and the only reaction that occurs is tagging by exchange of tritium for hydrogen. This was most gratifying as opposed to finding the major portion of bound tritium in radiolysis impurities in the Wilzbach method. In compounds studied so far, only isooctane exhibited some formation (about 0.5%) of new side products upon treatment with the tagging reagent. These side products are easily distinguished and removable by preparative chromatography.

It is interesting to note in Table III that *cis* and *trans* decalin do not tag at identical rates. Also note that naphthalene was tagged in cyclohexane solution (10%) to facilitate contact with the reagent.

Some preliminary kinetic data on tagging with the $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ are presented in Figures 2 to 5. Figure 2 compares the tagging rates of three aromatic compounds under similar conditions. Tetralin and toluene tag much more rapidly than benzene. This is due to the molecular electronic directional influence of the substituted groups on the benzene ring which activates aromatic hydrogen exchange.

It is seen from Figure 4 that anisole tags even more rapidly than tetralin or toluene. This is in line with the expected stronger electronic directional influence of the ether substituted benzene ring than the methyl substituted ring.

Figure 3 clearly illustrates the strong temperature dependence of tagging rate as observed for benzene. Thus tagging to equilibrium can be accelerated in cases which may be relatively slow.

Attention was then turned to tagging solids, including coal and derived products. Some results are shown in Figure 6. The coal extract, derived by hot tetralin extraction of a Pittsburgh Seam coal (Ireland Mine) is solid at the tagging temperature of 23°C. The amount of tritium exchange to the extract is obviously quite low, which is at least partly due to the fact that it must be predominantly a surface reaction. The coal itself (Ireland Mine) as shown in Figure 6, tags at an even slower rate than the extract. The reason for the lower rate is not clear though it may be a reflection of more isotopic or molecular diffusion in the extract.

Another sample of Pittsburgh Seam coal, but from the Montour No. 4 mine, was used to study the tagging rate over an extended period of time, i.e., 120 hours. These results are in Figure 7. A fresh sample of coal was used to reduce effects of oxidation. The coal also had been extracted with boiling methanol to remove any resinous materials from the coal particle surface that might inhibit contact with the labeling reagent. The results show that the coal continues to be exchange-labeled over a long period, probably by tritium diffusion through the solid coal. The theoretical limit (explained later) of labeling all of the 4.53% hydrogen content of this coal is 585 uc/gm as compared to only 30 uc/gm attained in 120 hours.

Solid naphthalene was tagged in the same way as coal with the results also shown in Figure 6. It tags even more slowly than coal. However, when naphthalene is tagged in the form of a 10% solution in cyclohexane, the tritium exchange is very rapid as is seen in Figure 6. It is apparent that the slow rate of tagging of coal and extract is primarily due to slow tritium diffusion through the solid and not necessarily connected with any deficiency of exchangeable aromatic hydrogen, since the aromatic naphthalene also tags very slowly in the solid state.

Discussion

Products of Wilzbach Tagging

It is clear that the chromatograph is not a sufficient criterion for radio-chemical purity of tracers produced by the Wilzbach method from polycyclic

hydrocarbons. The results indicate that many of the tagged products, from naphthalene, for example, are unstable tritium addition products rather than the desired isotopic exchange products. Some of these, as ditritio derivatives, may be unseparable chromatographically from naphthalene. Both the labile character of the tritium as seen by their ease of removal with sodium and the suggested polymerization in the chromatograph (hold-up), would fit the properties of compounds of this type.

It may be concluded that the Wilzbach tagging method is unsuitable for the production of reliable tracers from polycyclic aromatic compounds. Required post-tagging purification is too extensive.

Equilibrium Tagging With the $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ Reagent

A postulated mechanism of exchange labeling of an aromatic with the reagent was illustrated in Figure 1. It is likely that the powerful acidic properties of the complex cause formation of carbonium ions, accompanied by a proportional probability of adding a tritium ion to the organic compound. This meta-stable ion can then lose a hydrogen ion, yielding the tritium tagged compound.

The calculation of the theoretical limit of tagging is based on the assumption that at equilibrium the tritium is statistically distributed between all exchangeable hydrogen positions in the compound being tagged and in the phosphoric acid complex. This leads to the following expression for the tracer specific activity (S_x^∞) obtained at equilibrium, or ultimate labeling, as a function of initial specific activity of the reagent (S_r°), the weight ratio of tracer to reagent (w_x/w_r) and the number of exchangeable hydrogen positions (N_H) in the tracer.

$$S_x^\infty = \frac{S_r^\circ}{\frac{3}{166} \frac{M_x}{N_H} + \frac{w_x}{w_r}} \quad (1)$$

M_x is the molecular weight of the tracer compound.

As seen in Figure 3, the experimental limit of tagging of benzene at 65°F coincides well with the theoretical limit calculated from Equation (1), where $w_x/w_r = 0.5$ and taking $N_H = 6$. Though not given in this figure, long-term tagging studies of benzene have shown that the same equilibrium specific activity is reached in 80 hours at 23°C.

In Figure 5, it is seen that tagging of toluene at 23°C is approaching the calculated limit of tagging for five hydrogen positions but not for eight, i.e., all the aromatic positions are being tagged but not those on the methyl group. If tagging were being restricted to only the ortho and para aromatic hydrogens, then the limit would be that calculated for 3 hydrogens. Since this is exceeded by the experimental curve, the meta hydrogens must also be tagging, but likely at a lower rate than the ortho and para types.

Similarly, in Figure 2, the results with tetralin show the experimental limit to agree with the calculated limit for four exchangeable hydrogen positions which again corresponds to tagging of all of the aromatic positions only.

Anisole tagging, as shown in Figure 4, again behaves like that of toluene. The comparison of experimental limit of tagging with those calculated for 5 or 8 exchangeable hydrogens again indicates that only the 5 aromatic hydrogen positions are labeled and not the 3 on the methyl group.

Firmer conclusions can be reached from equilibrium tagging relative to the number of exchangeable positions by using a large excess of tritiating reagent. This improves the resolution between levels of tagging when different numbers of exchangeable hydrogen are considered. If $w_r \gg w_x$, then equation (1) reduces to the expression

$$S_x^{\infty} = S_r^{\infty} \left(\frac{166}{3} \right) \frac{N_H}{M_x}$$

and the equilibrium specific activity is thus proportional to the number of exchangeable hydrogens. On the other hand if $w_r \ll w_x$, then the specific activity of the compound becomes independent of N_H . Future work will therefore be carried out to confirm the number of exchangeable positions by the use of a large excess of tritiating reagent.

For the weight ratio used in this preliminary work, $w_x/w_r = 0.5$, the equilibrium tagging limit is not very sensitive to the number of exchangeable hydrogen positions. The relative values for S_x^{∞} for toluene for 3, 5 and 8 exchangeable positions with the above weight ratio are 1:1.1:1.15, whereas using an excess of reagent would spread the relative values of S_x^{∞} to 1:1.67:2.67.

Preliminary Examination of Exchange Reaction Kinetics

The kinetics of the isotopic exchange reaction with the tritiating reagent may be discussed using the mechanism shown in Figure 1 as a basis. It may be assumed that the opposing reaction rates are first order with respect to the concentration of the exchangeable hydrogen positions in the tracer and to the concentration of tritium in the tritiating reagent. Since we are often dealing with a two-phase system, absolute rate constants cannot be obtained. This is true since the rate is undoubtedly a function of the amount of interfacial contact area between the hydrocarbon and the phosphoric acid phases. Meaningful comparative rate data can be obtained, however, if constant conditions of agitation and a constant ratio w_x/w_r is employed in a series of comparable experiments.

It is known from prior work on deuterium exchange that aromatic hydrogens in different positions relative to orienting substituents on the benzene ring undergo exchange at considerably different rates. The rate equation for aromatic exchange can therefore be written in generalized form as

$$\frac{dS_x}{dt} = \sum_i k_i \left[S_r \left(\frac{N_{H_i}}{M_x} \right) - S_{x_i} \left(\frac{N_{H_r}}{M_r} \right) \right] \quad (2)$$

Subscript i denotes the various types of exchangeable hydrogen and S denotes specific activity. The second term on the right side of the equation corresponds

to the retardation effect of the reverse process, i.e., exchange of tritium from the compound with hydrogen in the reagent. It is assumed that the ratio of tritium to hydrogen is equal in both the reagent and compound after equilibrium is established and thus the rate constants of the forward and back reaction are equal.

The integration of equation (2) becomes rather complex when the specific activity of the reagent decreases during the course of the reaction. The initial rate can be used if desired to obtain a "weighted" average rate for all the exchangeable hydrogens

$$\frac{dS_x}{dt} = S_r \sum_i k_i \left(\frac{N_{H_i}}{M_x} \right) \quad (3)$$

However, due to the wide variation in rates between different exchangeable hydrogens the calculation of such an average rate would have little significance.

It is necessary to use the integrated form of equation (2) to derive values for the individual rate constants. To facilitate such integration, future kinetic data will be obtained under conditions where a large excess of reagent is employed, i.e., where S_r may be treated as constant. Integration of equation (2) becomes rather simple under such circumstances and leads to the expression

$$S_x = \frac{S_r M_r}{N_{H_r}} \sum_i \frac{N_{H_i}}{M_x} \left(1 - e^{-k_i \frac{N_{H_r}}{M_r} t} \right) \quad (4)$$

Though no data were yet taken with such an excess of reagent, sufficiently extensive data were obtained on benzene to permit calculation of rate constants in this particular case without having S_r constant. Since benzene has only one type of hydrogen, integration of equation (2) is rather simple in spite of the fact that S_r varies with time under the conditions of our experiments. The result is

$$\ln \left(\frac{1}{1 - S_x/S_x^\infty} \right) = k \left(\frac{N_H}{M_x} \right) \left(\frac{S_r}{S_x^\infty} \right) t \quad (5)$$

To derive values of the rate constants one merely has to plot the logarithmic term on the left versus time. This has been done for the benzene rate data at various temperatures and the results are given in Figure 8. The best straight lines were drawn through the data by the method of least squares. The fit to straight lines is rather good as seen. For example, the statistically

calculated correlation coefficients for the data at 21 and 45°C are 0.969 and 0.993 respectively. The values of the rate constants, k , are

$k (hr^{-1})$	$t (°C)$
0.16	21
2.19	45
7.93	65

The apparent activation energy, calculated from an Arrhenius plot of only three points of rate constant versus temperature, cannot claim much accuracy but yields a value of about 17,700 cal/mol.

A comparison may be made with recent rate data by Olssen¹⁰ on the rate of isotopic exchange between benzene and tritiated 80% H_2SO_4 acid at 25°C. The rate constant for the BF_3 reagent is greater by a factor of 200. The rate constants in heterogeneous systems of this type are, of course, not strictly comparable. Yet, the comparison shows, at least qualitatively, the very powerful nature of the $TH_2PO_4 \cdot BF_3$ reagent for promoting isotopic exchange.

Advantages of the $TH_2PO_4 \cdot BF_3$ Labeling Method

Many advantages of this new method of labeling with the tritiating reagent, $TH_2PO_4 \cdot BF_3$, have been demonstrated, as compared to the radiation induced self-labeling method. A summary of these follows:

1. Virtually radio-chemically pure tracers, without highly tagged side products, are produced in many cases. This obviates the extensive after-purification required in the radiation method. One has more faith in the tracer produced.
2. It is more rapid. The desired labeling can often be accomplished in a few hours as compared to several days by the latter method.
3. It is less involved. Ordinary chemical laboratory ware will suffice instead of special vacuum trains and gas handling systems.
4. It is less hazardous from a radiological health standpoint, because it avoids using multicurie amounts of radioactive gas (tritium) with the inherent possibilities of leakage in gas handling systems.
5. High specific activity tracers can be produced when desired. Tracer activity is easily controlled by the amount of tritium one chooses to incorporate in the reagent and the ratio of reagent to tracer compound mixed for labeling. The tagging reagent is very simple to manufacture from inexpensive tritiated water. It is a non-volatile liquid and need not incorporate much more tritium than wanted in the final tracer. Tracer specific activities of over a curie per gram can be produced when desired for experimental studies that will result in high dilution.

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Table I

Results of Tritium Tagging by Wilzbach Method For Model Compounds
According to Dual Radiation - Mass Chromatography

Material	Tritium Activity In Irradiation (Curies)	Irradiation (Curie-Hrs)	Specific Activity		Percent in Radiolysis Impurities
			Non- Purified Product	In Parent Compound	
Decalin	10	2620	387	204	47.2
Tetralin	8.1	2100	242	155	35.8
Naphthalene	9.4	2680	745	647	13.1
Phenanthrene	5.9	2420	79.9	79.9	0.2
Dihydrophenanthrene (60%)	6.1	1317	118	112	5

Table II

Naphthalene Tritiated by Willzbach Method

<u>Purification Treatment</u>	<u>Resultant Specific Activity (uc/gm)</u>
None	80.0
Na Treatment of Benzene Solution	56.0
Recrystallization From Benzene	53.9
Recrystallization From Methanol	50.4
Fractional Distillation	26.0
Complete Purification	?

Table III

Tritium Tracers Produced With $\text{TH}_2\text{PO}_4 \cdot \text{BF}_3$ at 23°C

<u>Labeled Compound</u>	<u>Reaction Time (Hrs)</u>	<u>Specific Activity Before Purification (uc/gm)</u>	<u>Specific Activity, Purified Tracer (uc/gm)</u>
Benzene	6.0	106.6	108.2
Toluene	6.5	550.0	554.0
Tetralin	5.5	536.0	531.0
Naphthalene	7.0	1422.0	1440.0
Anisole	6.0	146.0	146.0
Methylcyclohexane	6.0	9.25	9.36
Decalin	6.0	32.2	31.4
cis - Decalin	6.0	-	52.5
trans - Decalin	6.0	-	23.8
Cyclohexane (99.6%)	6.0	0.96	0
n-Octane (98.6%)	6.0	16.3	0

Figure 1

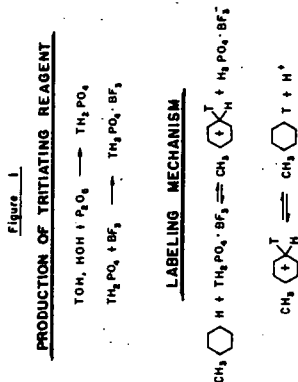


Figure 2

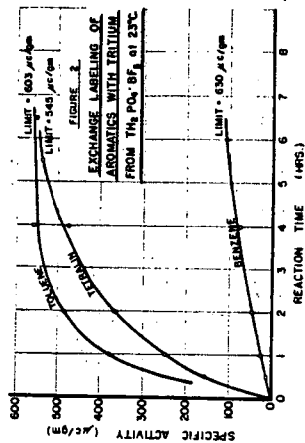


Figure 3

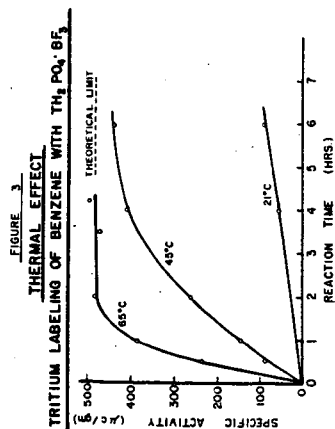


Figure 4

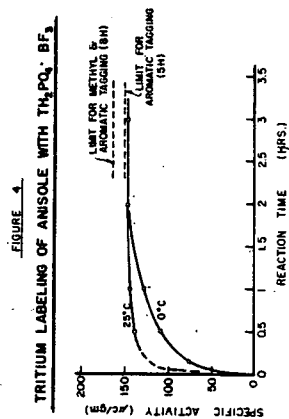


Figure 5

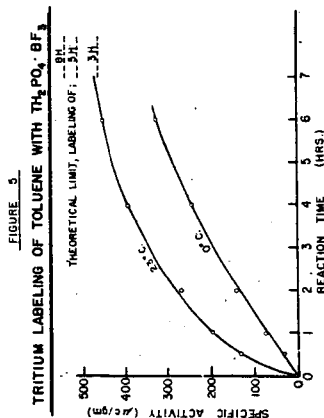


Figure 7

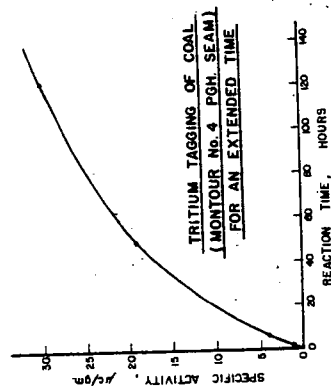


Figure 6

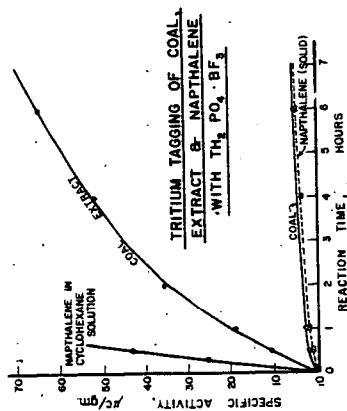


Figure 8

